U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-300 459

Biological Screening of Complex Samples from Industrial/Energy Processes

Research Triangle Inst, Research Triangle Park, NC

Prepared for

Industrial Environmental Research Lab, Research Triangle Park, NC

Aug 79

United States
Environmental Protection
Agency

Process Measurements Branch
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EPA-600/8-79-021
August 1979

EPA

Biological Screening of Complex Samples From Industrial/Energy Processes

Cooperative Project of IERL–RTP and EPA Health and Ecological Laboratories

PB 300459

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/8-79-021	2.
3. RECIPIENT'S ACCESSION NO. PB300459	
4. TITLE AND SUBTITLE Biological Screening of Complex Samples from Industrial/Energy Processes	5. REPORT DATE August 1979
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) N. G. Sexton	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Research Triangle Institute P.O. Box 12194 Research Triangle Park, North Carolina 27709	10. PROGRAM ELEMENT NO. 1AB604 and 1BB610
	11. CONTRACT/GRANT NO. 68-02-2688, Task 104
12. SPONSORING AGENCY NAME AND ADDRESS EPA, Office of Research and Development Industrial Environmental Research Laboratory Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED Special; 9/78 - 7/79
	14. SPONSORING AGENCY CODE EPA/600/13

15. SUPPLEMENTARY NOTES IERL-RTP project officer is Raymond G. Merrill, Mail Drop 62, 919/541-2557.

16. ABSTRACT

The report describes a cooperative project between EPA/IERL-RTP and EPA health and ecological laboratories to establish a biological screening program for complex samples from industrial and energy processes. The report summarizes the program elements and their application to various complex environmental samples. Results from the application of this program show that it is effective for screening complex mixtures. The report also describes the data formatting procedures used to report results from a diversity of biological tests in a meaningful way. It also compares the biological screening approach to proposed programs from other EPA program offices: the comparison shows a similarity in the approach for biological screening within EPA.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution	Pollution Control	13B
Bioassay	Stationary Sources	06A
Tests	Biological Screening	14B
Industrial Processes		13H
Energy Processes		10B

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 24
	20. SECURITY CLASS (This page) Unclassified	22. PRICE A02-A01

EPA Form 2220-1 (9-73)

Biological Screening of Complex Samples From Industrial/Energy Processes

A Cooperative Project of IERL-RTP and EPA Health and Ecological Laboratories

Process Measurements Branch
Industrial Environmental Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC
June 1979

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BIOLOGICAL SCREENING OF COMPLEX SAMPLES FROM INDUSTRIAL/ENERGY PROCESSES

INTRODUCTION

The earliest indicators of the dangerous nature of an unknown substance were biological. Sudden fish kills often indicated point source contamination of water supplies. In Japan, a plastics factory discharging wastes containing mercury into Minimata Bay ultimately caused 46 human deaths and poisoned another 120 persons. In a highly industrial society, it is essential that these hazardous discharges be detected and controlled before they cause gross and sometimes irreversible damage to the environment. The biological testing scheme incorporated as part of the EPA/IERL-RTP's environmental assessment procedures is designed for this purpose.

The biological testing protocols discussed in this report are the result of a cooperative effort by personnel from the health and ecological effects laboratories of the Environmental Protection Agency working with personnel from IERL-RTP. The cooperative programs were initiated in 1974. In 1976, a subcommittee representing EPA health and ecological laboratories was formed. Members of this subcommittee, listed in Table 1, prepared the test protocols and evaluated results obtained on complex process samples from three pilot studies. These studies were completed in 1978.

THE PHASED APPROACH TO ENVIRONMENTAL ASSESSMENTS

EPA's Industrial Environmental Research Laboratory at Research Triangle Park (IERL-RTP) has developed an environmental assessment scheme for the detection of hazardous materials in industrial effluents and gaseous emissions. IERL-RTP's environmental assessment scheme incorporates five major components: technology background development; sampling and analysis; environmental goals; impact analysis; and control technology evaluation. The sampling and analytical scheme couples a battery of biological indicators with a series of chemical tests to characterize the nature of a stream in terms of hazardous materials. This scheme involves three levels of effort; Level 1 is a screening phase for the detection of any effluent or emission that might be detrimental to the environment; Level 2 involves verification of the hazardous properties, indicated by Level 1 tests, and specific identification of chemical components of the stream; and Level 3 is a long-term monitoring phase, initiated to determine variations of the hazardous materials in an industrial stream with time and to evaluate the efficiency of control technologies. Note that an indication of possible hazard in Level 1 chemistry or biology dictates proceeding to Level 2, where positive results are confirmed and false positives screened out. Level 3 then monitors selected chemical and biological indicators over a period of time. Figure 1 graphically presents the phased approach.

TABLE 1. ENVIRONMENTAL ASSESSMENT STEERING COMMITTEE: BIOASSAY SUBCOMMITTEE MEMBERS

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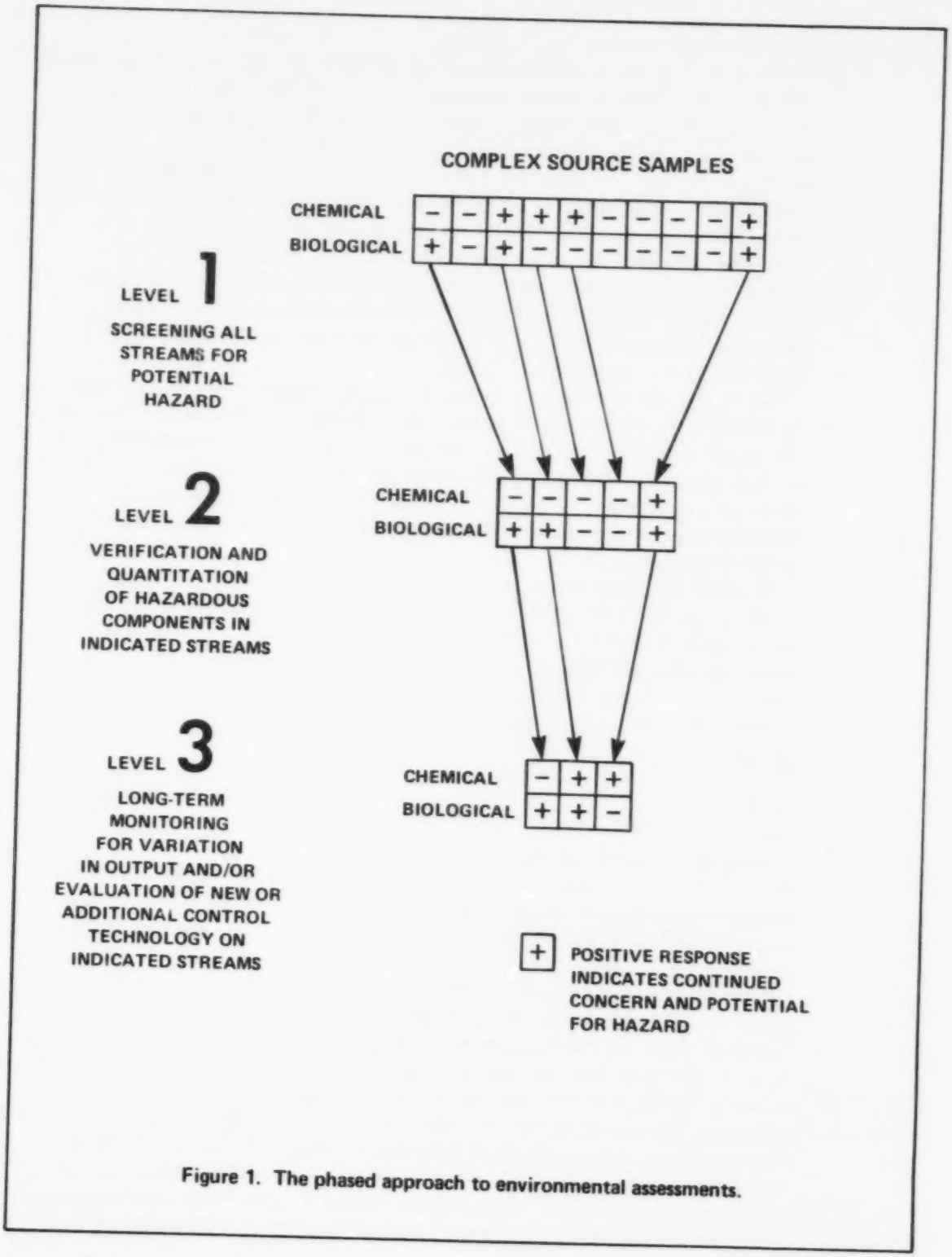
COMPLEX SOURCE SAMPLES
CHEMICAL
BIOLOGICAL
LEVEL 1
SCREENING ALL STREAMS FOR POTENTIAL HAZARD
LEVEL 2
VERIFICATION AND QUANTITATION OF HAZARDOUS COMPONENTS IN INDICATED STREAMS
LEVEL 3
LONG-TERM MONITORING FOR VARIATION IN OUTPUT AND/OR EVALUATION OF NEW OR ADDITIONAL CONTROL TECHNOLOGY ON INDICATED STREAMS
+ POSITIVE RESPONSE INDICATES CONTINUED CONCERN AND POTENTIAL FOR HAZARD

Figure 1. The phased approach to environmental assessments.

LEVEL 1 BIOLOGICAL TESTS

The Level 1 screening phase uses a series of short-term bioassays for the detection of acute biological effects (reference 1). The bioassays can be divided into health-related tests and ecological tests. The health tests were selected to screen for both acute toxic and potential chronic (i.e., carcinogenic) health effects. The rodent acute toxicity test employs a quantal prescreen in rats followed by a quantitative assay. The cellular toxicity assay in mammalian cells measures cellular viability or survival as well as more sensitive metabolic endpoints. The cells employed in Level 1 are rabbit alveolar macrophage (RAM) for particulates, and Chinese Hamster Ovarian (CHO) or WI-38 human embryonic lung cells for liquids. Also included in the health tests is the Ames *Salmonella typhimurium* reverse mutation assay. This assay uses bacteria to screen complex process samples for potentially mutagenic or carcinogenic activity.

The aquatic ecological tests measure acute toxic effects of an industrial effluent or emission on freshwater or marine fish (fathead or sheepshead minnows), freshwater or marine invertebrates (*Daphnia* or grass shrimp), and selected species of freshwater or marine algae. The aquatic ecological tests are "static" tests, involving the exposure of the various organisms to several concentrations of an effluent sample in a closed system or aquarium. The organisms are monitored for abnormal responses or death.

Terrestrial ecological tests, including soil respiration, root elongation, plant stress ethylene, and insect tests, are under evaluation; these tests are being developed and will be part of the Level 1 protocol in the near future. The soil respiration test is based on changes in normal uptake or output of carbon dioxide by soil microbes when a solid or liquid toxic material is introduced into the soil. The root elongation test evaluates samples via the inhibitory effect of toxic chemicals on seed germination and root elongation. The stress ethylene test assays samples by way of the release of ethylene by plants when the plants are exposed to toxic gases. The insect test will measure the toxicity of solid, liquid, or gaseous samples on sensitive insect species.

Although no single biological test can thoroughly predict the overall hazardousness of a material, this battery of rapidly run biological tests on a wide variety of biological receptors or indicators can indicate the kind of damage a material could produce. Table 2 details the Level 1 biological tests. Figure 2 is a matrix of the application of each Level 1 biotest to specific sample types.

The Level 2 biological tests are being developed to verify or confirm the Level 1 presumptive positive results and give more specific information on the nature of the biological effect. As will be illustrated in the following section on case studies, the Level 1 screening bioassays will prioritize streams for further testing so that the available resources of time and funding can be directed toward the relatively few hazardous streams. Where Level 1 chemical or biological tests indicate the need for further analysis, Level 2 biological tests can be applied to more clearly define the health or ecological hazard related to the stream in question. In some cases, Level 2 biological tests may be as simple as those in Level 1, while other cases will require much more elaborate and classical methods for assessing biological hazard.

TABLE 2. LEVEL 1 SCREENING BIOASSAYS

HEALTH EFFECTS TESTS

TEST	EFFECT	DESCRIPTION	TEST OUTPUTS
Microbial Mutagenesis (Ames Test)	Mutagenesis	Genetically sensitive strains of microorganisms are exposed to various doses of sample with and without metabolic activation.	Mutagenic response is measured relative to controls.
Cytotoxicity	Cellular Toxicity	Selected cells (RAM, CHO, or WI-38) are exposed to various doses of sample, then various endpoints are measured.	An index of functional impairment, toxicity, and metabolic change is established relative to controls.
Rodent Acute Toxicity (RAT Test)	Whole Animal Toxicity	Rats or other rodents are fed a quantity of sample, then observed daily for adverse symptoms over a 14-day period. The experiment is terminated with a necropsy exam.	Inventory of pharmacological and gross physiological effects in a whole animal system.

ECOLOGICAL EFFECTS TESTS

TEST	EFFECT	DESCRIPTION	TEST OUTPUTS
Algal Growth Response	Algal Growth Inhibition or Promotion	Cultures of selected marine and/or freshwater algae are used to gauge reaction to sample or dilution thereof.	Growth response measure—stimulation or inhibition.
Aquatic Animal Exposure (Static Acute Bioassay)	Animal Toxicity	Select marine and/or freshwater vertebrates and invertebrates are exposed to a graded dilution series of samples.	Gross index of toxic potential to representative animals.
Terrestrial	Toxicity to Plants, Insects, and Soil Microbiota	Tests in these three areas are under evaluation.	Effects on plants, insects, and soil of complex samples.

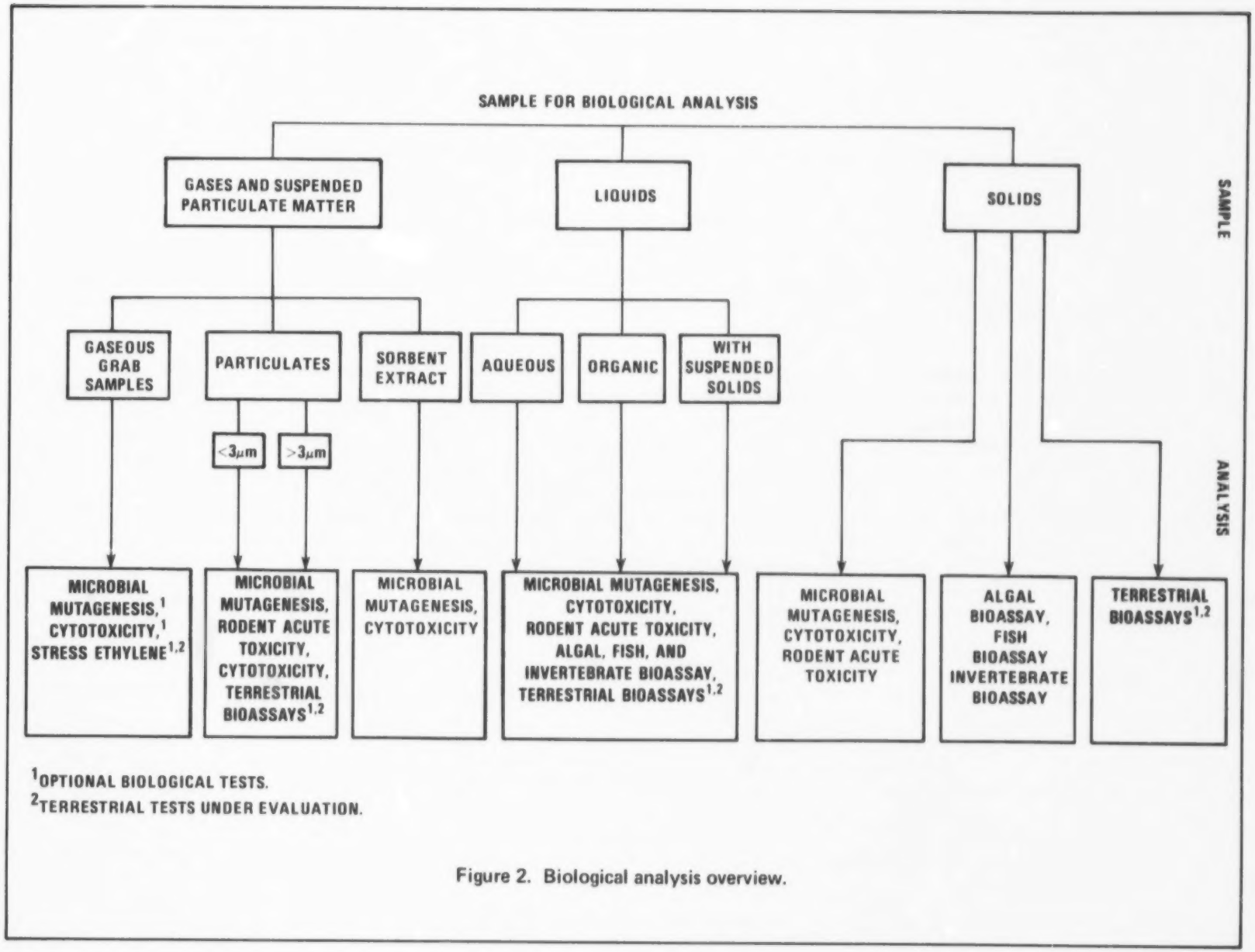

Figure 2. Biological analysis overview.

LEVEL 1 CHEMICAL TESTS

The Level 1 sampling and analysis plan calls for simultaneous collection and testing for biological and chemical parameters. The Level 1 chemical testing plan uses spark source mass spectrometry (SSMS) for qualitative and semiquantitative elemental analysis and ion chromatography (IC) for anion analysis. Gas chromatography (GC), liquid chromatographic (LC) separation of organic components into similar groups, gravimetric determinations, infrared spectroscopy (IR), and low resolution mass spectrometry (LRMS) are employed for characterization of organic compounds in a sample. As in the biological testing scheme, the Level 1 chemical testing scheme is designed to produce a semiquantitative and qualitative characterization of a material. The more costly and time-consuming Level 2 chemical tests are quantitative and compound-specific, and these Level 2 tests are employed where Level 1 tests indicate a potential for environmental detriment. Figure 3, from the revised Level 1 Procedures Manual (reference 2), illustrates the combined biological and chemical testing plan on solids, slurries, and liquids.

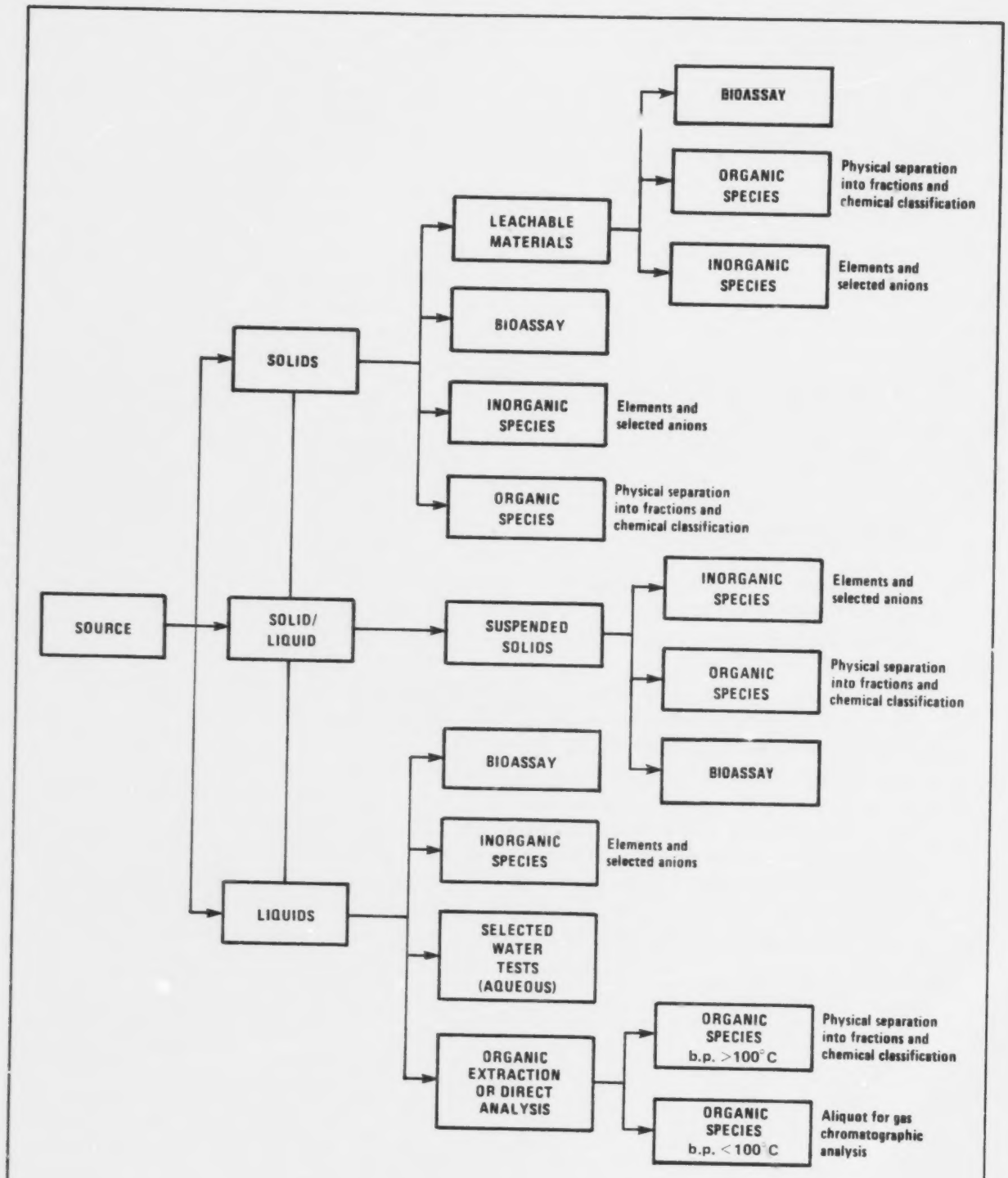

Figure 3. Basic Level 1 sampling and analytical scheme for solids, slurries, and liquids.

SUMMARY AND RANKING OF THE RESULTS OF ENVIRONMENTAL ASSESSMENTS

The combined biological and chemical screening scheme produces a large number of individual test results, and EPA/IERL-RTP has recognized a need to summarize these results and numerically relate them to some degree of severity or hazard. In order to summarize the *chemical data,* a list of threshold levels or minimal concentrations of concern has been prepared for a large number of chemical compounds. This list is generally based on whole animal tests of toxic materials and, more specifically, on LD_{50} (lethal dose to 50 percent of a population) testing performed by oral administration of various substances to rodents (reference 3). If a particular chemical compound or chemical group, in the worst case, exceeds the minimum level of concern, then Level 2 testing is indicated.

A Source Assessment Model (SAM) (reference 4) has been devised for tabulating the Level 1 chemical data. In this model, the Level 1 chemical data are formatted, and chemical concentrations are ratioed to levels of concern. These ratios are totaled, giving discharge severities (DS). The rate of discharge of an effluent is factored in, and total discharge severity rates (DSR) are calculated.

Summarizing the biological data from such a wide variety of test types is a formidable task. Methods for introducing the complex *biological data* into this matrix of information have recently been proposed by EPA/IERL-RTP (reference 5). These methods are based on the maximum applicable dose (MAD) for each biological test, which is the maximum amount of a substance that can be administered in a given bioassay.

The MAD concept must be applied differently to each biological test. Response is ranked as high, moderate, low, or nondetectable. Table 3 gives response ranges and maximum applicable doses for several of the Level 1 bioassays.

In the Ames test, the criteria for a positive (mutagenic) response is a ratio of test revertants to control revertants greater than 2 for Strains TA1535, TA1537, TA1538, TA98, or TA100, plus an increasing dose/response relationship over three successive dose ranges.

TABLE 3. RESPONSE RANGES FOR RANKING OF VARIOUS BIOTESTS

ASSAY	ACTIVITY MEASURED	MAD	RESPONSE RANGES			
			HIGH	MODERATE	LOW	NOT DETECTABLE
Health Tests						
Ames	Mutagenesis	5 mg/plate or 500 μL/plate	<0.05 mg or <5 μL	0.05-0.5 mg or 5-50 μL	0.5-5 mg or 50-500 μL	ND at >5 mg or ND at >500
RAM, CHO, WI-38	Lethality (LC_{50})	1,000 μg/mL or 600 μL/mL	<10 μg or <6 μL	10-100 μg or 6-60 μL	100-1,000 μg or 60-600 μL	LC_{50} >1,000 μg or LC_{50} >600 μL
Rodent	Lethality (LD_{50})	10 g/kg or 10 mL/kg	<0.1	0.1-1.0	1-10	LD_{50} >10
Ecological Tests						
Algae	Growth Inhibition (EC_{50})	1,000 mg/L or 100%	<20% or <200 mg	20-75% or 200-750 mg	75-100% or 750-1,000 mg	EC_{50} >100% or EC_{50} >1,000 mg
Fish	Lethality (LC_{50})	1,000 mg/L or 100%	<20% or <200 mg	20-75% or 200-750 mg	75-100% or 750-1,000 mg	LC_{50} >100% or LC_{50} >1,000 mg
Invertebrate	Lethality (LC_{50})	1,000 mg/L or 100%	<20% or <200 mg	20-75% or 200–750 mg	75-100% or 750-1,000 mg	LC_{50} >100% or LC_{50} >1,000 mg

MAD = Maximum Applicable Dose (Technical Limitations)
LD_{50} = Calculated Dosage Expected to Kill 50% of Population
LC_{50} = Calculated Concentration Expected to Kill 50% of Population
EC_{50} = Calculated Concentration Expected to Produce Effect in 50% of Population
ND = Not Detectable

CASE STUDIES: EXAMPLES OF LEVEL 1 OUTPUTS

Some examples of actual chemical and biological test results from Level 1 Environmental Assessments illustrate Level 1 outputs and the applications of these outputs. Three pilot studies were run to evaluate the Level 1 methodologies, and the data shown here are drawn from these pilot studies. The studies involved assessments of a coal gasifier, a series of textile plants' effluents, and a fluidized-bed combustor (references 6, 7, and 8). Figure 4 shows the biological results for samples chosen from the textile effluent pilot study to illustrate a range of biological responses; this figure also shows a DS value for health and for ecology for each sample. These DS values were calculated by summing the ratios of each chemical test result to a threshold level of concern for that chemical category. Table 4 presents the health-related bioassay results from the pilot studies by decreasing DS-health value, and Table 5 shows the ecologically related bioassay results ordered by decreasing DS-ecological value. The most hazardous samples, based on chemical analysis, are at the top of the lists. Because none of these samples caused a lethal response in rats but some samples caused abnormal behavioral or physiological responses, Table 4 also presents a sum of the number of abnormal responses. Figure 5 shows how the bioassays can be used to rank samples; in this figure, all samples are ranked for their cytotoxicity in RAM cells.

Several conclusions can be drawn from examination of the chemical and bioassay results of these samples.

1. There was a total of 175 bioassays run on these 27 samples as shown in Tables 4 and 5. The Level 1 bioassay screening procedures indicated that 110 of these (63 percent) showed no detectable toxicity. This screening procedure eliminates the need for costly and time-consuming Level 2 bioassaying in many instances.
2. Relatively high chemically based health-related DS values usually occur in samples with positive bioassays. Notable exceptions to this pattern do occur. For example, referring to Table 4, Plant X had a relatively low health-related DS value but gave positive indication of cell toxicity in the RAM test.
3. There also is a correlation between the chemically derived ecological DS values and ecologically related bioassays, but exceptions to this pattern of interpretation also occur. For example, Plant W ranked 24th out of 27 samples for ecologically related, chemically based DS values, but Plant W gave low or moderate responses on five of six ecological bioassays. Where differences in the chemical model and the biological tests occur, the analysis may not be measuring the same effect. Therefore, chemical tests and biological assays complement each other and should be run in parallel.
4. The Discharge Severity Model, based on *chemical* measurements, shows a fair correlation between ecologically related DS values and health-related DS values. Those samples showing a high potential for health-related damage also generally show a high potential for ecologically related damage.
5. The DS Model also shows a fair degree of agreement between health and aquatic ecological tests; however, the ecological tests appear to be more sensitive than the chemical model as presently performed for liquid samples.

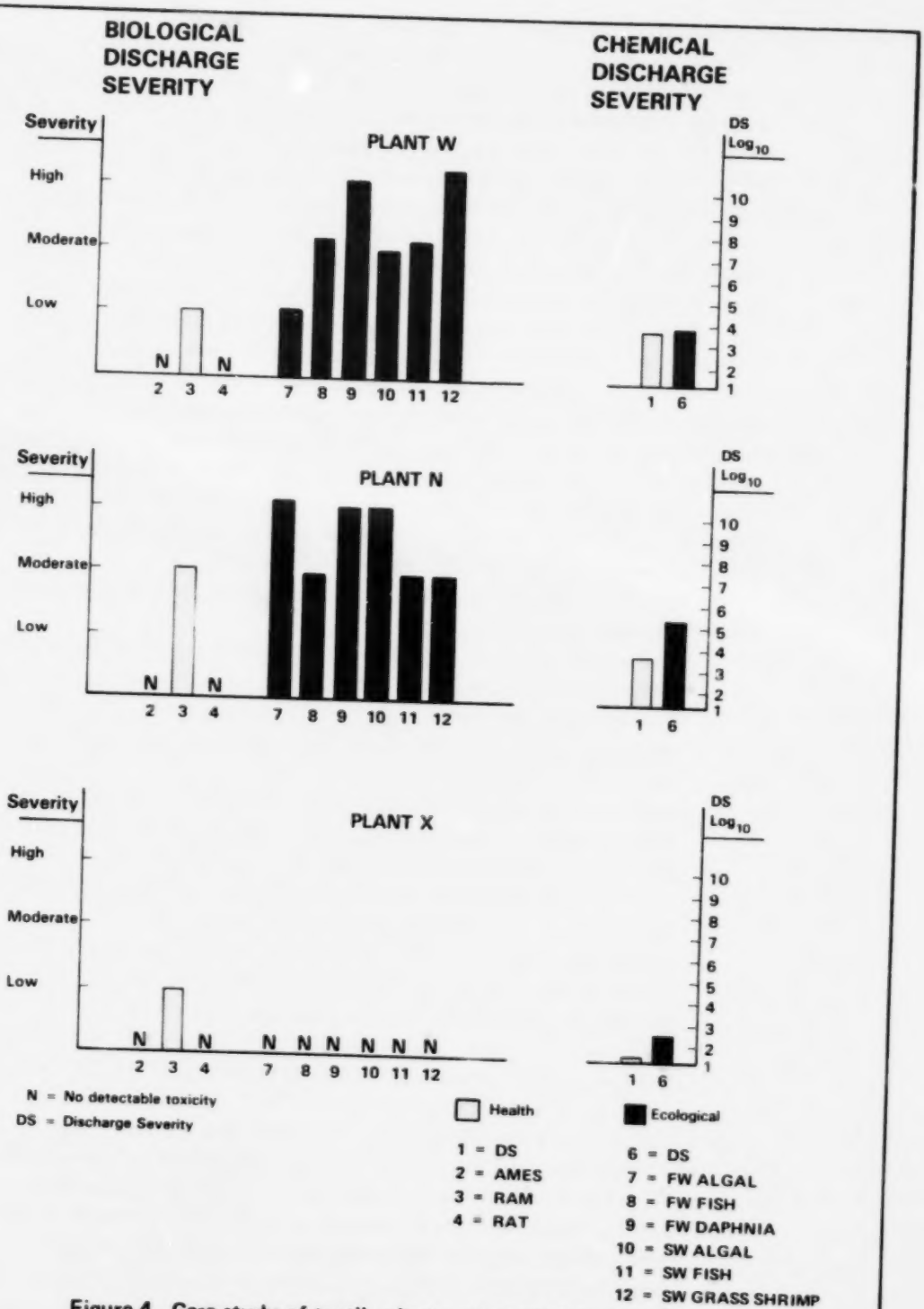

Figure 4. Case study of textile plants: selected biological and chemical test results.

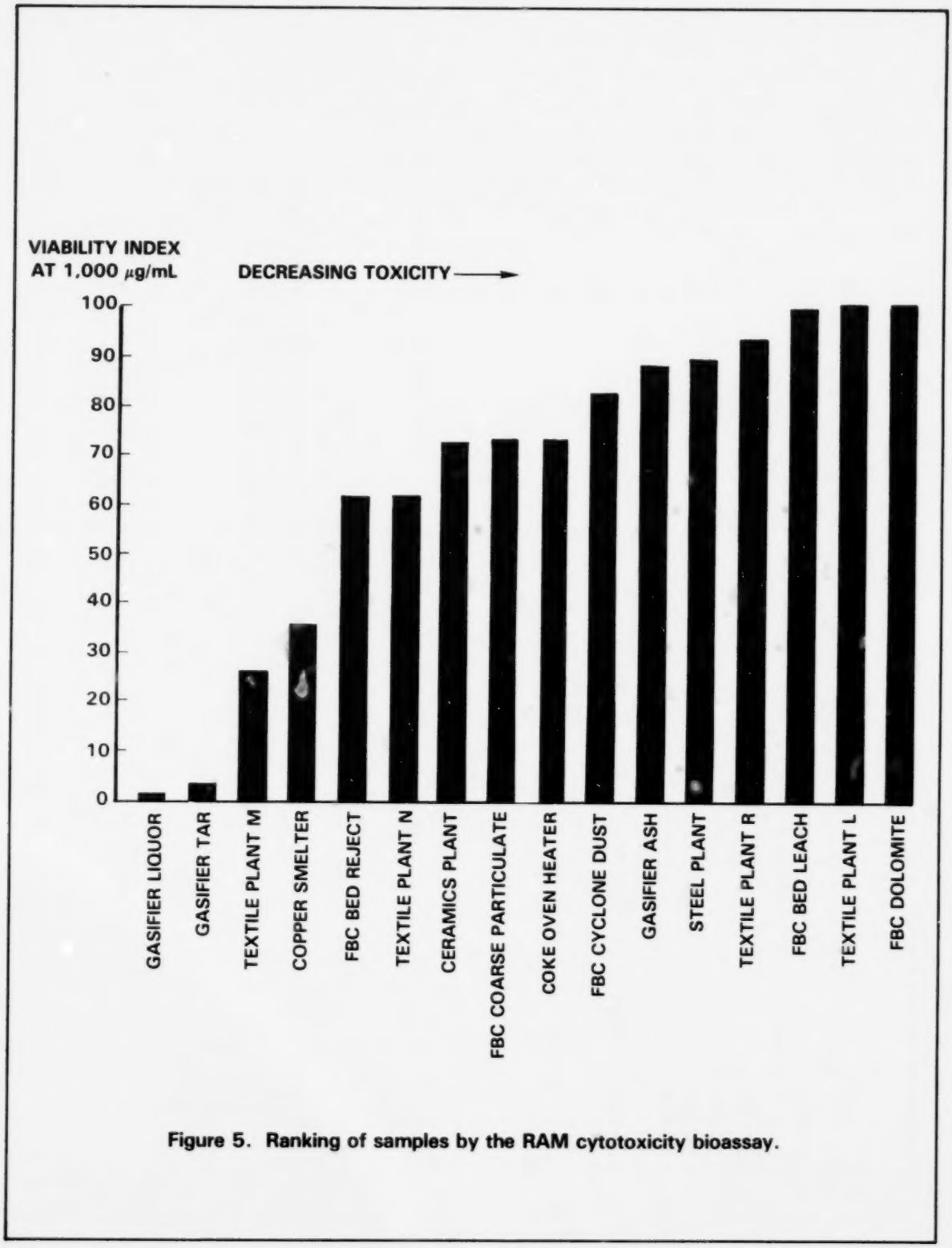

Figure 5. Ranking of samples by the RAM cytotoxicity bioassay.

TABLE 4. HEALTH EFFECTS: SUMMARY OF CHEMICAL AND BIOLOGICAL TEST RESULTS

STUDY	SAMPLE	1 DS	2 AMES	3 RAM	4 RAT	5 RAT – ABNORMAL RESPONSES
Coal Gasifier	Separator Liquor	6.1×10^{7}	N	M	N	0
Fluidized-Bed Combustor	Bed Reject Leachate	1.2×10^{6}	*	N	*	*
Coal Gasifier	Separator Tar	1.0×10^{6}	H	L	*	*
Textile Effluent	Plant L	8.4×10^{3}	N	M	N	5
Textile Effluent	Plant W	5.0×10^{3}	N	L	N	0
Textile Effluent	Plant N	3.3×10^{3}	N	M	N	6
Coal Gasifier	Gasifier Ash	1.7×10^{3}	N	N	N	0
Textile Effluent	Plant U	8.9×10^{2}	N	N	N	0
Fluidized-Bed Combustor	Fine Particulates	7.0×10^{2}	L	L	*	*
Textile Effluent	Plant F	4.5×10^{2}	N	L	N	4
Fluidized-Bed Combustor	Cyclone Dust	3.8×10^{2}	N	N	*	*
Textile Effluent	Plant B	3.3×10^{2}	N	N	N	6
Fluidized-Bed Combustor	Bed Reject	1.6×10^{2}	N	N	*	*
Textile Effluent	Plant G	9.4×10^{1}	N	N	*	*
Textile Effluent	Plant X	3.6×10^{1}	N	L	N	0
Textile Effluent	Plant E	3.3×10^{1}	N	N	N	0
Fluidized-Bed Combustor	Dolomite	2.3×10^{1}	N	N	*	*

* = Data Not Available
H = HIGH Toxicity Rating
M = MODERATE Toxicity Rating
L = LOW Toxicity Rating
N = No Detectable Toxicity

1 = DS = Discharge Severities
2 = AMES = Mutagenicity Test
3 = RAM = Rabbit Alveolar Macrophage
4 = RAT = Rodent Acute Toxicity
5 = RAT – Abnormal Responses = Sum of the Number of Sublethal Abnormal Behavioral and Physiological Responses Noted in the 10 Rats Used in Testing Each Sample

TABLE 5. ECOLOGICAL EFFECTS: SUMMARY OF CHEMICAL AND BIOLOGICAL TEST RESULTS

STUDY	SAMPLE	6 DS	7 FW ALGAL	8 FW FISH	9 FW DAPHNIA	10 SW ALGAL	11 SW FISH	12 SW GRASS SHRIMP
Fluidized-Bed Combustor	Bed Reject Leachate	1.5×10^8	M	M	M	*	N	N
Coal Gasifier	Separator Liquor	6.9×10^7	H	H	H	H	H	H
Fluidized-Bed Combustor	Fine Particulates	1.0×10^6	*	*	*	*	*	*
Coal Gasifier	Separator Tar	2.9×10^5	*	*	*	*	*	*
Textile Effluent	Plant N	1.5×10^5	H	M	H	H	M	M
Textile Effluent	Plant L	6.1×10^4	M	M	M	M	N	N
Textile Effluent	Plant B	3.0×10^4	M	N	N	N	N	N
Textile Effluent	Plant E	2.9×10^4	H	N	H	*	N	N
Fluidized-Bed Combustor	Cyclone Dust	2.6×10^4	*	*	*	*	*	*
Textile Effluent	Plant F	2.2×10^4	N	N	L	L	N	N
Textile Effluent	Plant U	1.5×10^4	N	N	H	N	N	N
Textile Effluent	Plant G	1.2×10^4	N	M	M	M	N	N
Coal Gasifier	Gasifier Ash	9.3×10^3	*	*	*	*	*	*
Textile Effluent	Plant W	5.6×10^3	L	M	H	M	M	H
Fluidized-Bed Combustor	Bed Reject	2.7×10^3	*	*	*	*	*	*
Fluidized-Bed Combustor	Dolomite	4.8×10^2	*	*	*	*	*	*
Textile Effluent	Plant X	3.4×10^2	N	N	N	N	N	N

* = Data Not Available
H = HIGH Toxicity Rating
M = MODERATE Toxicity Rating
L = LOW Toxicity Rating
N = No Detectable Toxicity

6 = DS = Discharge Severities
7 = FW ALGAL = Freshwater Algal
8 = FW FISH = Fathead Minnow
9 = FW DAPHNIA = Freshwater Invertebrate
10 = SW ALGAL = Marine Algal
11 = SW FISH = Marine Sheepshead Minnow
12 = SW GRASS SHRIMP = Saltwater Shrimp

CURRENT STATUS OF THE LEVEL 1 BIOLOGICAL SCREENING PROGRAM

The matrix of bioassays in Level 1 will continue to be reviewed and evaluated. The following activities are being evaluated for possible inclusion in the screening matrix:

- Modifications to the rodent acute toxicity protocol to increase sensitivity.
- Additional Level 1 screening tests for chronic health effects (i.e., carcinogenic).
- Concentration and extraction methods for the Ames microbial mutagenesis assay.
- Comparison and selection of optimum cellular toxicity assays.
- Evaluation and incorporation of soil, plant, and insect tests into the terrestrial bioassay group.
- Evaluation of chemical fractionation procedures compatible with bioassays.
- Development of bioassays for testing gaseous samples.
- Development of quality control procedures specific to Level 1 tests.

The health and ecological labs are also working closely with IERL-RTP on joint interpretation of chemical/biological results.

BIOASSAY PROGRAMS IN OTHER EPA LABORATORIES

This cooperative biological testing program parallels the development of similar programs in several other EPA laboratories and is consistent with more recent development of similar programs within the Agency that stress multiple biological endpoints in their test matrixes. The Office of Toxic Substances, the Office of Pesticide Programs, the Office of Solid Wastes, the Environmental Monitoring and Support Laboratory in Cincinnati (EMSL-Cin), and the Ocean Dumping Permits Office all require or propose to require bioassaying programs for the evaluation of hazardous materials. Table 6 compares the Level 1 biological testing program with the proposed or draft bioassay requirements of these other program offices.

TABLE 6. COMPARISON OF IERL-RTP LEVEL 1 BIOASSAY REQUIREMENTS WITH THOSE OF EPA PROGRAM OFFICES

BIOLOGICAL TEST	IERL RTP LEVEL 1	OFFICE OF SOLID WASTE	OFFICE OF TOXIC SUBSTANCES (ToSCA)[1,2]	OCEAN DUMPING PERMITS	EMSL CIN (EPA SUBCOMMITTEE)	OFFICE OF PESTICIDE PROGRAMS[1,2]
In Vitro Mutagenicity or Carcinogenicity	Bacterial Mutagenicity	Bacterial Mutagenicity Mammalian Somatic Cells in Culture[3] Fungal Microorganisms[3] DNA Repair in Bacteria[3] Unscheduled DNA Repair in Human Diploid Cells[3] Mitotic Recombination or Gene Conversion in Yeast[3] Sister Chromatid Exchange in Mammalian Cells[3]	Mutagenic Oncogenic			Bacterial Mutagenicity[4] Eucaryotic Microorganism Mutagenicity[4] Mammalian Somatic Cells, Mutagenicity[4] Bacterial DNA Repair[3] Mammalian Cells, Unscheduled DNA Synthesis[3] Yeast, Mitotic Recombination or Gene Conversion[3] Sister Chromatid Exchange[3]
In Vitro Cytotoxicity	Rabbit Alveolar Macrophage (For Particulate) WI-38 or CHO Cloning (For Solutions)		Subacute and Chronic Toxicity (6 Tests Suggested)			
Whole Animal	Quantal Range Finding		Epidemiological Studies Teratogenic Oncogenic Reproductive			Mammalian Cytogenetics[5] Insect, Heritable Chromosomal Effects[5] Rats, Dominant Lethal[5] Rodents, Heritable Translocation[5] Oncogenic (2 mammalian species) Mouse, Specific Locus Test[4] Insect, Sex-Linked Recessive Lethal[4]
Freshwater Ecological	Fathead Minnow Algae Daphnia Magna	Daphnia Reproduction			Fathead Minnow Algae Daphnia	
Marine Ecological	Sheepshead Minnow Algae Grass Shrimp			Sheepshead Minnow[6] Algae[6] Grass Shrimp[6] Mysid Shrimp[6] Ascartia Tonsa[6]		
Terrestrial Ecological	Soil Respiration[7,10] Insect[7,10] Root Elongation[7,10] Stress Ethylene	Germination Seedling Growth	Germination Seedling Growth			

[1] The open wording of the Toxic Substances Control Act permits the possibility of including data from a wide variety of short term tests as part of the test data requirements.
[2] Efforts are underway to combine the requirements for bioassay for the Office of Toxic Substances and the Office of Pesticide Programs.
[3] These tests have been proposed but are not yet legally required.
[4] Proposed requirement of a minimum of three tests, but not more than one test of each type mentioned.
[5] Choose one of the tests in this group to partially satisfy disposal requirements.
[6] Choose two of the tests in this group to partially satisfy disposal requirements.
[7] Proposed requirement of a minimum of two tests, but not more than one test of each type mentioned.
[8] Proposed requirement of a minimum of three tests, but not more than one test of each type mentioned.
[9] These tests are done in both short and long term modes.
[10] These tests are optional until validated.

CONCLUSIONS

Chemical tests alone cannot adequately characterize the hazardous nature of a complex material. A scarcity of adequate information on the biological effects of pure compounds exists in many instances; and, even if such information were available, the present level of knowledge on synergistic effects and antagonistic effects is inadequate to predict the response of a biological system to an effluent or emission composed of many chemical compounds. When high levels of a known hazardous chemical compound are present in a sample, a strong biological response can usually be anticipated, but the converse cannot be assumed. As illustrated in the section on case studies, biological responses were noted in cases where chemical DS rating was relatively low.

The present level of knowledge and the currently available information point toward the cost-effective use of a Level 1 screening program that uses complementary bioassays and chemical tests for predicting environmental hazard. EPA/IERL-RTP has used the described screening program in its ongoing environmental assessment program with considerable success. Experience in the use of chemistry-bioassay screening methodology common to many R&D programs has allowed refinements of the tests to provide cost-efficient reliable assessments of environmental impact from a wide variety of source and sample types.

It should be reiterated that the health and ecological labs are working closely with IERL-RTP on joint interpretation of chemical/biological results. The cooperative program will continue to evaluate and revise the Level 1 biological protocols in order to incorporate new developments in the area. For example, short-term subacute and chronic tests applicable to Level 1 screening bioassays are being reviewed.

In addition, the subcommittee is in the process of structuring a Level 2 protocol which will include subacute and chronic effects tests and/or fractionation of samples for verification and quantification of results from the screening studies.

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This paper was prepared by Research Triangle Institute under contract #68-02-2688 with the Industrial Environmental Research Laboratory of the Environmental Protection Agency.